Identification of the \textit{in Vitro} HIV-2/SIV RNA Dimerization Site Reveals Striking Differences with HIV-1*

Received for publication, September 21, 2000, and in revised form, November 10, 2000
Published, JBC Papers in Press, November 22, 2000, DOI 10.1074/jbc.M008642200

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Although their genomes cannot be aligned at the nucleotide level, the HIV-1/SIVcpz and the HIV-2/SIVsm viruses are closely related lentiviruses that contain homologous functional and structural RNA elements in their 5'-untranslated regions. In both groups, the domains containing the trans-activating region, the 5'-copy of the polyadenylation signal, and the primer binding site (PBS) are followed by a short stem-loop (SL1) containing a six-nucleotide self-complementary sequence in the loop, flanked by unpaired purines. In HIV-1, SL1 is involved in the dimerization of the viral RNA, \textit{in vitro} and \textit{in vivo}. Here, we tested whether SL1 has the same function in HIV-2 and SIVsm RNA. Surprisingly, we found that SL1 is neither required nor involved in the dimerization of HIV-2 and SIV RNA. We identified the NarI sequence located in the PBS as the main site of HIV-2 RNA dimerization. cis and trans complementation of point mutations indicated that this self-complementary sequence forms symmetrical intermolecular interactions in the RNA dimer and suggested that HIV-2 and SIV RNA dimerization proceeds through a kissing loop mechanism, as previously shown for HIV-1. Furthermore, annealing of tRNA\textsubscript{3} to the PBS strongly inhibited \textit{in vitro} RNA dimerization, indicating that, \textit{in vivo}, the intermolecular interaction involving the NarI sequence must be dissociated to allow annealing of the primer tRNA.

Based on phylogenetic analysis, the HIV-1/SIVcpz and the HIV-2/SIVsm primate viruses belong to the same lentivirus genus of the retrovirus family (1). Furthermore, they cause similar diseases characterized by long and variable incubation periods, persistent viral replication, neurological manifestations, and destruction of specific hematologic and immunologic cells (for a review see Ref. 2). The HIV-1/SIVcpz and the HIV-2/SIVsm possess equivalent accessory proteins, and although their genomes cannot be aligned with each other at the nucleotide level, their leader regions contain equivalent functional and structural RNA elements (3).

In HIV-1,\textsuperscript{1} the trans-activating region stem-loop, the hairpin containing the polyadenylation signal, and the structural domain containing the primer binding site (PBS) are followed by four short hairpins involved in genomic RNA dimerization, splicing of the viral RNAs, packaging of the genomic RNA, and initiation of gag translation, respectively (see Fig. 1A) (3). A similar situation also prevails in HIV-2 and SIVsm genomic RNAs (Fig. 1B). In those viruses, six short hairpins follow the first three structural domains. The third and the sixth short stem-loops contain the major splice donor site and the initiation codon of the gag gene, respectively, whereas the second, the fourth, and the fifth are parts of the packaging signal (3). Interestingly, the first of the short HIV-2/SIVsm stem-loops (SL1) contains a loop with a six-nucleotide self-complementary sequence flanked by one or more purines, as does the HIV-1/SIVcpz DIS loop (see Fig. 1) (3, 4). Thus, it is appealing to hypothesize that SL1 fulfills similar functions in HIV-1/SIVcpz and in HIV-2/SIVsm (3, 4).

\textit{In vitro} experiments identified the HIV-1 SL1 as the dimerization initiation site (DIS) of the genomic RNA (5), and it is now widely accepted that dimerization is initiated by symmetric intermolecular interactions between the self-complementary sequences of the loop (5–19). Indeed, mutations affecting the self-complementarity of the DIS loop inhibited dimerization (5, 7, 8, 19), whereas the introduction of complementary mutations restored the process (7, 8). Furthermore, the unpaired purines flanking the self-complementary sequence appeared to modulate the dimer stability (7, 8, 10, 19).

When infectious HIV-1 molecular clones were mutated in the DIS, the replication rate of the viruses dramatically decreased (18, 20, 21), and their infectivity was reduced by two to three orders of magnitude (21–24). A significant fraction of the genomic RNA inside the viral particles was monomeric (18), and the residual dimer appeared abnormal when assessed by nondenaturing electrophoresis (25), even though the thermal stability of the dimers of mutated RNAs was similar to that of the wild-type dimer (20, 22). Mutations in the DIS affected at least two steps of the HIV-1 replication cycle: encapsidation of the genomic RNA (20–22, 26–28), and reverse transcription (21, 24).

As dimerization of genomic RNA is ubiquitous among retroviruses (29–42), and the location and structural features of the HIV-1 DIS and the HIV-2/SIVsm SL1 are conserved (see Fig.

\textsuperscript{*} This work was supported by a grant from the Agence Nationale de Recherche sur le SIDA (ANRS). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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\textsuperscript{1} The abbreviations used are: HIV-1 and -2, human immunodeficiency virus types 1 and 2; DIS, dimerization initiation site; PBS, primer binding site; SIV, simian immunodeficiency virus; SL1, stem-loop 1; PCR, polymerase chain reaction; UTR, untranslated region; NCp, nucleocapsid protein.
1), it has been proposed that SL1 might be involved in the
dimerization of the HIV-2/SIVsm genomic RNA (3, 4). How-
ever, unlike that of HIV-1, dimerization of the HIV-2/SIVsm
genomic RNA has received little attention, and the sequences
governing this process have not been precisely identified (43).

Thus, we decided to test whether SL1 is involved in the
dimerization of the HIV-2/SIVsm genomic RNA, in vitro. In
this study, we showed that, despite its similarity to the HIV-1 DIS,
the SL1 of HIV-2 ROD and SIV MM239 is neither required for
nor involved in RNA dimerization. Furthermore, we identified
a self-complementary sequence located in the primer binding
site (PBS) that is required for the dimerization of the HIV-2
ROD and SIV MM239 RNAs. Interestingly, tRNA\textsubscript{3}L\textendash\textasciitilde\textasciitilde
annealing to the PBS prevented in vitro RNA dimerization. The
implications of this finding for the mechanism of RNA dimerization
and tRNA annealing in vivo are discussed.

MATERIALS AND METHODS

Template Construction for in Vitro Transcription—A sense primer
containing an EcoRI site and the promoter for the T7 RNA polymerase
(GAA TTT TAC GAC TCA CTA TAG CTC GCT CGG AGA
GCG TGG) and an antisense primer (GAA TTC AGT TTC TCG CGC
CCA TCT CCC) were used to amplify the 550 first nucleotides of SIV
MM239 RNA. HIV-2 SL1 PCR product was generated using primers GGAATTCTA-TATACGACTCTAGCTCTAGCTAGAGCGC (sense) and TCCCC-
C GGCTCCACACGCTGC (antisense).

RNA was synthesized and purified as described (7), except RNAs
compacting the HIV-2 SL1 and the HIV-1 DIS, which were purified
from the unincorporated nucleotides using a Centricon 10 (Amicon).
These RNAs were 5′-end-labeled for 30 min at 37 °C with \( γ \)\textasciitilde\textasciitilde
ATP (3000 Ci/mmol) and T4 polynucleotide kinase.

RESULTS

Potential Role of SL1—Given the structural similarity be-
tween the HIV-1 DIS and SL1 in the HIV-2 and SIVsm genomic
RNAs (Fig. 1), our first experiments were designed to test the
involvement of SL1 in the in vitro dimerization of HIV-2 and
SIV RNAs. HIV-2 ROD and SIV MM239 were chosen as proto-
types of the human and simian immunodeficiency viruses,
respectively. These two viruses contain rather divergent SL1.
Indeed, the SL1 loop contains 11 nucleotides in HIV-2 ROD
RNA, but only 7 nucleotides in SIV MM239 RNA. However,
In Vitro Dimerization of HIV-2 and SIV RNA

**HIV-2 RNA**

A. HIV-2 RNAs 1–561 and 1–420 (550 nM) were incubated in monomer (lanes marked M) and dimer (lanes marked D) buffer. The monomeric (m) and dimeric (d) species were separated on an agarose gel and stained with ethidium bromide. B. The HIV-2 ROD SL1 were incubated in dimer buffer. The monomeric (m) and dimeric (d) species were separated on a nondenaturing polyacrylamide gel and revealed by autoradiography.

**SIV RNAs**

A. HIV-2 ROD RNAs 1–561 and 1–420 (550 nM) were incubated in monomer (lanes marked M) and dimer (lanes marked D) buffer. The monomeric (m) and dimeric (d) species were separated on an agarose gel and stained with ethidium bromide. B. The HIV-2 ROD RNAs used to localize the dimer linkage sequences; bottom, RNAs encompassing nucleotides 1–561, 1–420, 1–337, and 1–307 of HIV-2 ROD were incubated in monomer (M) and dimer (D) buffer. The monomeric (m) and dimeric (d) species were separated and stained as above.

**Fig. 2.** SL1 is not involved in the dimerization of HIV-2 ROD RNA. A, HIV-2 RNAs 1–561 and 1–420 (550 nM) were incubated in monomer (lanes marked M) and dimer (lanes marked D) buffer. The monomeric (m) and dimeric (d) species were separated on an agarose gel and stained with ethidium bromide. B, radioactively labeled 50-mer RNA corresponding to the HIV-1 DIS and 47-mer RNA encompassing the HIV-2 ROD SL1 were incubated in dimer buffer. The monomeric (m) and dimeric (d) species were separated on a nondenaturing polyacrylamide gel and revealed by autoradiography.

Both loops contain the same GGUACC self-complementary sequence (Fig. 1B).

To test the putative role of SL1 in the dimerization of HIV-2 RNA, we compared the salt-induced dimerization of RNAs encompassing nucleotides 1–561 or 1–420 of HIV-2 ROD (+1 corresponding to the transcription start). Although the larger RNA contains the complete 5′-untranslated region (5′-UTR) of HIV-2 ROD, the shorter one stops at the first nucleotide of the self-complementary sequence in the SL1 loop (Fig. 1). As observed with RNA fragments corresponding to 5′-UTR of other retroviruses, HIV-2 ROD RNA 1–561 was essentially monomeric under low salt conditions but efficiently dimerized in a buffer containing 50 mM sodium cacodylate, 300 mM KCl, and 5 mM MgCl₂ (Fig. 2A). Quite surprisingly, HIV-2 ROD RNA 1–420 also dimerized under high salt conditions, and its dimerization yield was comparable to that of HIV-2 ROD RNA 1–561 (Fig. 2A).

Similarly, we compared the dimerization of RNA fragments corresponding to nucleotides 1–549 or 1–417 of SIV MM239, the shorter one ending in the SL1 loop (Fig. 1). The salt-induced dimerization of SIV RNAs was somewhat less efficient than that of HIV-2 RNAs. However, as observed for HIV-2 RNAs, the dimerization efficiencies of the large and the short RNA were similar.

These results indicated that neither the SL1 hairpin structure, nor the self-complementary sequence in the SL1 loop were required for dimerization of HIV-2 ROD and SIV MM239 RNAs. However, the possibility remained that, although not absolutely required, SL1 was nevertheless involved in one way or another in the dimerization process. To test this possibility, we synthesized a 47-mer RNA containing the HIV-2 SL1, and compared its salt-induced dimerization with that of a 50-mer RNA containing the HIV-1 DIS (Fig. 2B). In agreement with previous observations (6, 10), the HIV-1 DIS dimerized efficiently, even at the rather low RNA concentration used in this assay. On the contrary, no dimer could be detected when using the HIV-2 SL1 (Fig. 2B). The lack of dimerization of the HIV-2 SL1 was not due to RNA misfolding, because chemical probing indicated that SL1 adopted the expected stem-loop structure, both in the 47-mer RNA and in the 1–561 HIV-2 RNA. Thus, our results indicated that SL1 was not involved in the dimerization of HIV-2 RNA.

**Dimerization of 3′-Truncated RNAs**—To identify the sequences involved in the dimerization of HIV-2 RNA, we synthesized RNAs gradually devoid of the 3′-part of the UTR by in vitro transcription of plasmid pSLROD linearized with EcoRI, KpnI, BbsI, or NarI, respectively. This allowed us to compare dimerization of RNAs encompassing nucleotides 1–561, 1–420, 1–337, and 1–307 of HIV-2 ROD (Fig. 3A). As described above, RNA 1–561 and RNA 1–420 dimerized efficiently under high salt conditions. In addition, we observed that RNA 1–337 dimerized with the same efficiency as those RNAs, whereas dimerization of RNA 1–307 was strongly reduced (Fig. 3A).

Similarly, we compared dimerization of RNAs corresponding to nucleotides 1–550, 1–419, and 1–308 of SIV MM239. Dimerization of the SIV RNA fragments was somewhat reduced, as compared with HIV-2. Nevertheless, we observed that RNA 1–550 and RNA 1–419 dimerized with a similar efficiency, whereas RNA 1–308 hardly dimerized (Fig. 3B). Thus, our deletion experiments indicated that the RNA stretch located between nucleotides 307 and 337 in HIV-2 ROD, and 308 and 419 in SIV MM239 is required for efficient in vitro dimerization of those RNAs.

2 F. Jossinet, unpublished results.
Site-directed Mutagenesis of the NarI Site in the PBS of HIV-2 RNA—In HIV-1 (4–12, 15, 16, 18–23, 25, 46), murine leukemia virus (33, 41, 47–50), and avian sarcoma and leukemia virus (36, 51), dimerization of the genomic RNA involves a self-complementary sequence. No such sequence is contained between nucleotides 307 and 337 of HIV-2 ROD RNA and presenting the NarI sequence (shaded) in the loop. The positions that were mutated are indicated by asterisks. The PBS is indicated by a gray double arrow. A, predicted intermolecular interactions for wild-type (left), C305 (middle), and C305G308 (right) HIV-2 RNA 1–561 if the NarI sequence forms a kissing loop complex. C, wild type, C305, and C305G308 HIV-2 RNAs 1–561 were incubated in monomer (M) and dimer (D) buffer. The monomeric (m) and dimeric (d) species were separated on an agarose gel and stained with ethidium bromide.

To test the role of the NarI sequence in the dimerization of HIV-2 ROD RNA, we mutated nucleotide G305 to C. If, as has been shown for HIV-1, HIV-2 RNA dimerizes by forming a kissing loop complex between self-complementary sequences, this substitution should destroy two out of six intermolecular base pairs (Fig. 4B). In agreement with this model, we observed that substituting C for G305 in HIV-2 RNA 1–561 dramatically reduced dimerization of that mutant RNA (Fig. 4C). According to the kissing loop model, substituting G for C305 should compensate for the C305 substitution, by restoring six intermolecular base pairs between the kissing loops (Fig. 4B). Indeed, we observed that the C305G308 compensatory HIV-2 RNA dimerized even more efficiently than the wild-type HIV-2 ROD RNA 1–561. Thus, our results strongly suggest that HIV-2 RNA dimerizes by a kissing loop mechanism involving the self-complementary NarI sequence.

However, one could not totally exclude that this sequence played an indirect role. The crucial point could be a particular structure of the NarI sequence in the monomer maintained by base pairing of nucleotides 305 and 308, which would be required for dimerization, without any interaction of these sequences in the dimer. To test this possibility, we investigated the capability of the C305G308 compensatory HIV-2 RNA to form heterodimers with the wild-type HIV-2 RNA. Because both RNAs are able to form homodimers, they should also be able to form heterodimers, if the interaction between nucleotides 305 and 308 is intramolecular. On the contrary, no heterodimer should be formed between these RNAs if this interaction is intermolecular. We used RNA of either 561 or 337 nucleotides in length to distinguish between homo- and heterodimers (Fig. 5). When wild type HIV-2 RNA 1–561 and 1–337 were mixed in low salt buffer, two main bands corresponding to the monomeric RNA species were observed. When these RNAs were coincubated in high salt buffer, three bands were observed (Fig. 5). They were attributed to the HIV-2 RNA 1–337 homodimer (bottom), the HIV-2 RNA 1–561 homodimer (top), and the HIV-2 RNA 1–337/HIV-2 RNA 1–561 heterodimer (middle). On the contrary, when C305G308 HIV-2 RNA 1–561 and wild-type HIV-2 RNA 1–337 were coincubated in the high salt buffer, only the two bands corresponding to the homodimeric species were observed (Fig. 5). Thus, this experiment suggested that the interaction between nucleotides 305 and 308 is intermolecular.

To further test the kissing loop model, and unambiguously prove that the NarI sequence is involved in intermolecular interactions, we examined the possibility of forming a heterodimer between C305 and G308 HIV-2 RNAs (Fig. 6). In this experiment, the heterodimer should be favored over the homodimers if the interaction between nucleotides 305 and 308 is intermolecular (Fig. 6A). To test this prediction, we used 32P-labeled G308 HIV-2 RNA 1–561 and unlabeled C305 HIV-2 RNA 1–561. As observed above with C305 HIV-2 RNA 1–561, we found that C305 HIV-2 RNA 1–561 hardly dimerized in the high salt buffer (Fig. 6B, lane 2). However, the fraction of G308 HIV-2 RNA 1–561 in the dimeric form strongly increased in the presence of unlabeled C305 HIV-2 RNA 1–561, indicating that these two RNAs efficiently formed heterodimers (Fig. 6B, lane 3). These results unambiguously demonstrated the direct intermolecular interaction, in the HIV-2 RNA dimer, between the NarI sequences located in the PBS of each monomer.

Effect of RNA5′″ Annealing on RNA Dimerization—Because
the Nar1 site is located within the PBS, we tested whether annealing of primer tRNA$_{3}^{\text{Lys}}$ interfered with RNA dimerization (Fig. 7). We first heat-annealed a mixture of $^{32}$P-labeled and unlabeled tRNA$_{3}^{\text{Lys}}$ purified from beef liver to the viral RNA by a 20-min incubation at 70 °C in a buffer lacking magnesium chloride, followed by a standard incubation in the dimerization buffer (see “Materials and Methods”) (Fig. 7A, lane 2). In a control experiment, the same thermal treatment was applied to the viral RNA, in the absence of tRNA$_{3}^{\text{Lys}}$ (Fig. 7A, lane 1). When tRNA$_{3}^{\text{Lys}}$ was omitted, wild type HIV-2 RNA 1–561 was found almost completely in the dimeric form, indicating that the thermal treatment per se did not inhibit RNA dimerization. However, dimerization of wild type HIV-2 RNA 1–561 was strongly inhibited in the presence of tRNA$_{3}^{\text{Lys}}$. Similarly, we observed that binding of tRNA$_{3}^{\text{Lys}}$ to the PBS of SIV RNA 1–550 strongly inhibited its dimerization (data not shown). On the other hand, tRNA$_{3}^{\text{Lys}}$ did not affect dimerization of C305G308 HIV-2 RNA 1–561 (Fig. 7A, lanes 3 and 4). Autoradiography of the gel indicated that tRNA$_{3}^{\text{Lys}}$ was bound to wild type but not to C305G308 HIV-2 RNA 1–561 (Fig. 7B). Furthermore, it showed that tRNA$_{3}^{\text{Lys}}$ was also bound to the small fraction of dimeric wild type HIV-2 RNA 1–561 (Fig. 7B, lane 2). These results supported the involvement of the Nar1 site in the dimerization of HIV-2 RNA, and they also showed that even though this site greatly favored this process, it was not strictly required for RNA dimerization. This result was in keeping with the fact that point mutations in the Nar1 site strongly decreased but did not totally inhibit dimerization (Figs. 4 and 6) and that HIV-2 RNA 1–307 weakly dimerized (Fig. 3).

DISCUSSION

The similar location of the HIV-2/SIVsm SL1 and the HIV-1/SIVcpz DIS and the presence of a self-complementary sequence in the loop of both hairpins led to the proposal that SL1 might be involved in the dimerization of the HIV-2/SIVsm genomic RNA (3, 4). However, experiments performed to test this hypothesis led to a different conclusion. Unexpectedly, SL1 is neither required nor involved in the dimerization of RNAs corresponding to the 5′-end of the HIV-2 ROD. Furthermore, even though most experiments were conducted with HIV-2 ROD-derived RNAs, the experiments performed with the SIV MM239-derived RNAs suggest that our conclusions also hold true for the latter virus. At first sight, the finding that SL1 is unable to dimerize despite the presence of a six-nucleotide self-complementary sequence in the loop is surprising. However, recent systematic evolution of ligands by exponential enrichment experiments on the HIV-1 DIS revealed that the presence of U and A at the central positions of the self-complementary sequence is strongly detrimental to dimerization (52).

Because SL1 is not involved in RNA dimerization, our data indicate that the HIV-2/SIVsm SL1 and the HIV-1/SIVcpz DIS are not totally functionally equivalent. However, mutations introduced in the DIS indicated a dual function for this hairpin. Indeed, although substitutions in the DIS loop and deletions of the DIS hairpin similarly prevent dimerization of HIV-1 RNA in vitro, encapsidation of the genomic RNA and viral replication are more severely affected by the latter mutations (21, 22, 25). The role of the DIS stem in RNA encapsidation is likely linked to its ability to bind the Gag precursor (53). However, the replacement of the DIS hairpin by an RNA aptamer, which binds Gag but does not dimerize, is unable to restore RNA packaging at wild type levels (54). On the other hand, SL1 is required for efficient packaging of HIV-2 RNA (55). Thus, SL1 performs only one of the two known DIS functions.

Our experiments unambiguously identified the Nar1 sequence located in the PBS as the primary dimerization site of...
HIV-2 and SIV RNAs. The reason why this site was not identified in a previous study using 3′-truncated HIV-2 RNAs is unclear (43). However, these authors did observe inhibition of HIV-2 RNA dimerization in the presence of large amounts of total yeast tRNA (43). The cis and trans complementation of mutations in the NarI sequence that we observed indicates that this sequence makes symmetric intermolecular contacts in the RNA dimer and suggests that HIV-2 (and SIV) RNA dimerizes by forming a kissing loop complex. Such a mechanism was previously demonstrated by complementary mutations in the case of HIV-1 (7, 8) and avian sarcoma and leukemia virus RNA (51) and is likely involved in the dimerization of murine leukemia virus RNA, even though, in this latter case, the dimerization mechanism is more complex (33, 48, 56, 57).

Several lines of evidence indicate that the PBS cannot promote the dimerization of HIV-1 RNA. An HIV-1 RNA encompassing R, U5, and the PBS, and truncated in the middle of the DIS self-complementary sequence does not dimerize (58). Similarly, HIV-1 RNAs containing the complete 5′-untranslated region and extending into the gag coding region do not dimerize if they contain deletions or substitutions in this self-complementary sequence (5, 7, 19), even though they contain the PBS. Furthermore, annealing of tRNA\textsuperscript{Lys} to the PBS does not inhibit HIV-1 RNA dimerization in vitro (59), indicating that the PBS is not required for the DIS-mediated HIV-1 RNA dimerization. Finally, deletion of the PBS does not impair RNA dimerization in vivo, and it does not affect the thermal stability of the dimer (60). Thus, the results obtained with HIV-1 indicate that the presence of a self-complementary sequence in the PBS is not sufficient for the PBS to be involved in RNA dimerization.

However, the involvement of the PBS sequence in RNA dimerization is not unique to HIV-2 and SIV. It has been previously described for bovine leukemia virus (38), which utilizes tRNA\textsuperscript{Pro} to prime reverse transcription. Because tRNA binding is compatible with RNA dimerization in retroviruses, tRNA\textsuperscript{Pro} annealing in bovine leukemia virus, HIV-2, and SIV may be a more sophisticated process than initially thought. We propose that, in vitro, dimerization of HIV-2 and SIVm RNA may be catalyzed by the establishment of intermolecular interactions all along the genomic RNA. These secondary interactions could take place either in the cytoplasm or during encapsidation and budding due to the annealing activity of the Gag precursor (62) (Fig. 8). The dimeric genomic RNA and the tRNA\textsuperscript{Lys} would be encapsidated by the NC domain of Gag (63) and the reverse transcriptase domain of Gag-Pol (64–66), respectively. The NC domain of the Gag precursor (62, 67), or the mature NCP (59, 68–70) would then disrupt the kissing loop interaction involving the NarI sequence of the PBS and anneal tRNA\textsuperscript{Lys} to this site, whereas the secondary RNA-RNA interactions would be maintained, therefore maintaining a dimeric RNA (Fig. 8).

Indeed, even in the well-documented case of HIV-1, there is no evidence that the initial interaction at the DIS is maintained in the mature dimer. Secondary interactions stabilizing the dimer are known to exist in vitro (7, 71), and likely take place in vivo during the NCP-induced maturation of the dimer (72, 73). Our finding that deletions and mutations of the NarI sequence or annealing of tRNA\textsuperscript{Lys} do not totally abolish HIV-2 RNA dimerization indicates that secondary interactions also exist in this RNA, which could be favored by the primary interaction at the NarI site. Because NCP is able to anneal almost any complementary sequences (74, 75), the role of the primary interaction could be to ensure that the NCP-mediated secondary interactions are not made randomly, but are properly oriented. Indeed, these interactions may be crucial for the strand-transfer events taking place during reverse transcription. This hypothesis is consistent with the observation that mutations in the HIV-1 DIS affect reverse transcription (21, 24).

The data presented in this paper reinforce the notion that the retroviruses fall into two classes depending on whether or not the PBS is involved in the dimerization of the genomic RNA. Remarkably, recent studies indicate that the yeast retrotransposons Ty1 (76) and Ty3 (77) belong to a third class, because dimerization of their genomic RNA was found to be mediated by the tRNA\textsuperscript{Pro} annealed to the PBS.

Acknowledgments—D. Mignot is acknowledged for skillful technical assistance. Thanks are due to J. C. Paillart for stimulating discussions and critical reading of this manuscript.

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